DISSERTATIONS IN
FORESTRY AND
NATURAL SCIENCES

Minna Hiltunen

The role of zooplankton in the trophic transfer of fatty acids in boreal lake food webs

Publications of the University of Eastern Finland
Dissertations in Forestry and Natural Sciences No 210

UNIVERSITY OF
EASTERN FINLAND

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Academic Dissertation
To be presented by permission of the Faculty of Science and Forestry for public examination in the Auditorium N100 in Natura Building at the University of Eastern Finland, Joensuu, on January, 15, 2016, at noon.

Department of Biology

Grano
Jyväskylä, 2015
Editors: Research Dir. Pertti Pasanen,
Profs. Pekka Kilpeläinen, Kai Peiponen, and Matti Vornanen

Distribution:
University of Eastern Finland Library / Sales of publications
P.O.Box 107, FI-80101 Joensuu, Finland
tel. +358-50-3058396
www.uef.fi/kirjasto

ISBN: 978-952-61-1632-7 (printed)
ISSNL: 1798-5668
ISSN: 1798-5668

ISBN: 978-952-61-1738-6 (PDF)
ISSNL: 1798-5668
ISSN: 1798-5676

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ABSTRACT:

The pelagic food webs of lakes produce fish that are valuable resources for humans because they are rich in essential polyunsaturated fatty acids (PUFAs). These PUFAs are synthesized by phytoplankton at the base of the food web and transferred to higher trophic levels via zooplankton. I studied the fatty acid composition of suspended particulate matter (seston), various zooplankton taxa, and planktivorous fish during three seasons in Finnish boreal lakes to elucidate how PUFAs are transferred through aquatic food webs, and ultimately become available for human consumption. Overall, seston in the studied lakes was rich in PUFAs, and thus a high quality resource for zooplankton. While seston was characterized by high proportions of C_{18} PUFAs, zooplankton and especially planktivorous fish had high proportions of the essential $C_{20\text{-}22}$ PUFAs. Majority of the variation in zooplankton fatty acid composition was related to taxonomy, but seasonal and dietary differences within taxa were also evident. More specifically, the fatty acid composition of zooplankton was connected to water chemistry, which most likely affects the phytoplankton community composition and thus the food for zooplankton. This study indicates that climate change-induced changes in temperature and water chemistry that may lead to shifts in phytoplankton and zooplankton community composition could cause changes in the dynamics of essential PUFAs in lake food webs.

Universal Decimal Classification: 543.635.353, 574.5, 574.583, 577.115.3

CAB Thesaurus: fatty acids; polyunsaturated fats; polyenoic fatty acids; lipids; zooplankton; phytoplankton; seston; organic carbon; freshwater fishes; lakes; food webs; trophic levels; taxonomy; variation

Yleinen suomalainen asiasanasto: rasvahapot; lipidit; plankton; kalat; sisävesikalat; järvet; ravintoverkot

Acknowledgements

This work would not have been possible without the help of a large group of people. First of all I would like to thank Paula Kankaala for giving me this opportunity even though I hardly knew what fatty acids were when I started. With secured funding for 3.5 years I could focus solely on the research. Your door was always open if I had any questions, and you helped me to see the big picture when I got caught up in the details.

In addition to Paula, I've had three other supervisors, and I am very grateful for the help of Ursula Strandberg, Sami Taipale and Raine Kortet. Ursula, we shared an office for several years and you never got tired of my endless questions, no matter how busy you might have been with other things. Sami, you taught me the basics of the lab analyzes, and have included me in many studies outside my thesis, and for that I am very grateful. Raine, you come from outside our fatty acid group and you gave me valuable perspective to the papers and to the thesis.

I can't thank enough the co-authors of the papers that form this thesis. I have learned a lot while writing the papers, and mostly because of your perceptive comments, and patience in guiding me through this process. My warm thanks goes to the whole staff of Department of Biology. I have always felt welcome here, and I've had so much fun working with you all. I want to thank all my fellow PhD students at the Universities of Eastern Finland, Jyväskylä, and Washington, and in the VALUE doctoral program for the peer support and friendly company. The field work that provided most of the data for this thesis would not have been possible without the help from our technical staff, and the crew on R/V Muikku. I would also like to thank all the students that have worked in the project over the years.

I gratefully acknowledge all the financial support received while working with my thesis. The whole project would not have happened without the funding from the Academy of Finland.

Most of the thesis summary was produced while I attended UW through Valle Scholarship Program, and when I got back to UEF the support from the faculty of Science and Forestry helped to finalize the thesis. Travel grants from the VALUE doctoral program have made it possible for me to attend international conferences and for that experience I am very grateful.

Last but not least I would like to thank all my friends and family for supporting me even though you sometimes did not have a clue what I was doing. You helped – and sometimes forced – me to take much-needed breaks from the work to recharge. Jukka, there are no words to express how much your support has meant to me. Thank you for always being there for me.

LIST OF ABBREVIATIONS

ALA	α-linolenic acid
ARA	Arachidonic acid
C_n PUFA	PUFA with *n* carbons
DHA	Docosahexaenoic acid
DOM	Dissolved organic matter
EPA	Eicosapentaenoic acid
FAME	Fatty acid methyl ester
FASTAR	Fatty acid source tracking algorithm in R
LIN	Linoleic acid
MUFA	Monounsaturated fatty acid
NMS	Non-metric multidimensional scaling
PLFA	Phospholipid fatty acid
POM	Particulate organic matter
PUFA	Polyunsaturated fatty acid
QFASA	Quantitative fatty acid signature analysis
SAFA	Saturated fatty acid
SDA	Stearidonic acid
tOM	Terrestrial organic matter
VLC-PUFA	Very long chain PUFA (> C_{22})

LIST OF ORIGINAL PUBLICATIONS

This thesis is based on data presented in the following articles, referred to by the Roman numerals I–V.

I Strandberg U, Taipale S J, Hiltunen M, Galloway A W E, Brett M T and Kankaala P. Inferring phytoplankton community composition with a fatty acid mixing model. *Ecosphere, 6: 16, 2015*.

II Hiltunen M, Strandberg U, Taipale S J and Kankaala P. Taxonomic identity and phytoplankton diet affect fatty acid composition of zooplankton in large lakes with differing dissolved organic carbon concentration. *Limnology and Oceanography, 60: 303-317, 2015.*

III Hiltunen M, Strandberg U, Keinänen M, Taipale S and Kankaala P. 2014. Distinctive lipid composition of the copepod *Limnocalanus macrurus* with a high abundance of polyunsaturated fatty acids. *Lipids, 49: 919-932, 2014.*

IV Hiltunen M, Taipale S J, Strandberg U, Kahilainen K K and Kankaala P. High intraspecific variation in fatty acids of *Eudiaptomus* in boreal and subarctic lakes. Submitted manuscript.

V Strandberg U, Hiltunen M, Jelkänen E, Taipale S, Kainz M, Brett M and Kankaala P. Selective transfer of polyunsaturated fatty acids from phytoplankton to planktivorous fish in large boreal lakes. *Science of the Total Environment, 536: 858-865, 2015*.

The above publications have been included at the end of this thesis with their copyright holders' permission.

AUTHOR'S CONTRIBUTION

The author contributed to the planning of studies in all the papers. The author was responsible for field work and laboratory analysis in the articles II and III, and collected part of the data in the articles I, IV and V. She did all the statistical analyses and wrote the papers II-IV, and participated in writing the papers I and V, jointly with the co-authors.

Contents

1 Introduction

1.1 STRUCTURE OF LAKE FOOD WEBS

Primary producers perform a fundamental role in food webs by providing energy and essential nutrients to higher trophic levels. In the pelagic zone of lakes, primary producers consist of phytoplankton that comprise numerous taxa of diverse phylogenetic origin. The autochthonous production by phytoplankton is grazed by zooplankton, which in turn, is used as a food source by fish larvae and planktivorous fish (Fig. 1). In addition to energy being channeled through this grazer food chain, terrestrial organic matter (tOM) can provide energy to higher trophic levels either directly or indirectly via the microbial loop (Tranvik, 1992). Dissolved organic matter (DOM), both from terrestrial and autochthonous sources, is a substrate for bacteria, which are grazed by heterotrophic nanoflagellates and ciliates (Fig. 1). In addition to phytoplankton, zooplankton graze on terrestrial particulate organic matter (tPOM), heterotrophic nanoflagellates and ciliates, and even bacteria making the energy of the microbial food web available for fish and other secondary consumers (Wenzel et al., 2012; Taipale et al., 2014).

The standing pools of OM in boreal lakes can be dominated by allochthonous sources with a large proportion of POM, and practically all of DOM derived from terrestrial sources (Wilkinson et al., 2013). Although the pool of tDOM can be large, the efficiency of energy transfer through the microbial food web is considered to be low because it involves multiple trophic steps (Berglund et al., 2007). The microbial loop can also be supported by algal-derived DOM, which has a very rapid turnover rate compared to tDOM, and supports higher bacterial growth efficiencies (Kritzberg et al., 2005). However, tDOM is a heterogeneous mix of molecules with widely varying bioavailability, and the labile parts (low molecular weight

compounds) can support as high growth rates in bacteria as phytoplankton-derived OM (Berggren et al., 2010). Thus, consumers in boreal lakes may rely on a dynamic mixture of autochthonous and allochthonous food sources (Pace et al., 2004; Kankaala et al., 2010; Scharnweber et al., 2014).

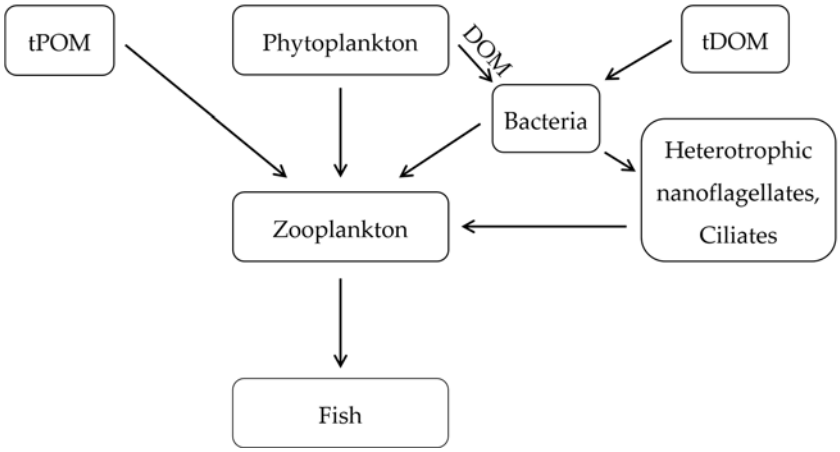

Figure 1. *Schematic figure of the structure of lake foodwebs. Modified from Jones (1992).*

1.2 FATTY ACIDS AS ESSENTIAL NUTRIENTS

The quality of food resources for consumers is determined by various factors, including size, shape, digestive resistance, toxicity and elemental and biochemical composition of the resource (Sterner, 1993; van Donk et al., 1997; Martin-Creuzburg & von Elert, 2004; Park et al., 2002; Becker & Boersma, 2005). Fatty acids are components of all lipids, and they consist of a straight or branched carbon chain, linked either with single or double-bonds, with a carboxyl group in one end. Besides being energy storage molecules, some fatty acids also have very important physiological functions. The fatty acid with 22 carbons, and six methylene-interrupted double bonds from the third carbon of the methyl end is called 22:6ω3, or docosahexaenoic acid (DHA). DHA is an omega-3 (ω3) polyunsaturated fatty acid (PUFA). PUFAs are important in the nutrition of all animals, including

humans. In cells, DHA is concentrated on the cell membrane and mitochondria, and it is abundant especially in the nervous tissue and retina of animals (Simopoulos, 1999; Stillwell & Wassal, 2003). Because of its important role in many metabolic functions, DHA is highly retained in consumers even during starvation (Castledine & Buckley, 1980; Kainz et al., 2010). High consumption of $C_{20\text{-}22}$ ω3 PUFAs in humans has been linked to reduced incidence of cardiovascular disease, arrhythmia, type 2 diabetes, cancer and depression (Simopoulos, 1999; Pelliccia et al., 2013). Furthermore, $C_{20\text{-}22}$ ω3 PUFAs improve cognitive development in infants (Jiao et al., 2014). Fish muscle contains high proportions of ω3 PUFAs eicosapentaenoic acid (EPA, 20:5ω3) and DHA, and thus fish are an important source of these essential fatty acids in human diets.

In addition to being essential for human nutrition, ω3 and ω6 PUFAs are important for aquatic consumers such as zooplankton and fish. Consumers differ in their composition and thus in their requirement of PUFAs (Persson & Vrede, 2006; Sargent et al., 1999; Tocher, 2010). It is generally accepted that essential fatty acids include the $C_{20\text{-}22}$ ω3 PUFAs EPA and DHA, and the ω6 PUFA arachidonic acid (ARA, 20:4ω6). Cladocerans are rich in EPA, while they have very little DHA, and the EPA content of their diet has been found to strongly affect the growth and reproduction of *Daphnia* (Müller-Navarra et al., 2000; Persson & Vrede, 2006). Furthermore, regardless of the levels in diet, the EPA concentration in *Daphnia* eggs is consistently higher than in body tissues, indicating that EPA is particularly important in *Daphnia* reproduction (Sperfeld & Wacker, 2011). Copepods contain high proportions of both EPA and DHA, and their egg production rates are higher when feeding on diets rich in these PUFAs (Burns et al., 2011; Jónasdóttir, 1994; Jónasdóttir et al., 1995; Evjemo et al., 2008).

There are some indications that ARA can also enhance *Daphnia* growth (Becker & Boersma, 2005), while other studies have found no evidence of ARA being a limiting fatty acid (von Elert, 2002; Ravet et al., 2012). In copepods, the proportion of ARA in the diet can be negatively correlated with egg hatching success (Evjemo

et al., 2008). In contrast to zooplankton, ARA clearly promotes fish growth, especially in larval fish (Cutts et al., 2012; Bell & Sargent, 2003). EPA and ARA are both precursors of eicosanoids, hormone-like local signaling molecules that affect inflammatory reactions, the ion transport system and reproductive processes in arthropods and fish (Stanley, 2000; Bell & Sargent, 2003). Studies suggest that fish need both ω3 and ω6 PUFAs in appropriate, species-dependent ratios to achieve normal ontogenetic development, maximal somatic growth rates, and reproduction (Sarget et al., 1999; Tocher, 2010). The optimum dietary ratio between ω3 and ω6 PUFAs is highly species specific, with silver perch (*Bidyanus bidyanus*) doing well on ratios < 1 (Smith et al., 2004), while maximal growth was obtained at ratios of 0.5-2 in rainbow trout (*Oncorhynchus mykiss*) and Atlantic salmon (*Salmo salar*) (Polvi and Ackman, 1992; Yang and Dick, 1994), at ~3 in pike (*Esox lucius*) (Engstrom-Öst et al., 2005) and at as high as 5-6 in Arctic char (*Salvelinus alpinus*) (Jónasson, 2008). Low levels of EPA, DHA or ARA in fish diets can lead to decreased stress tolerance, compromised parr-smolt transition in salmonids, abnormal pigmentation, and impaired vision leading to reduced growth and even decreased survival (Sargent et al., 1999; Bell & Sargent, 2003). Although the majority of studies on food quality effects on consumer growth have been conducted at non-limiting food quantities (e.g. Martin-Creuzburg & von Elert, 2004; Brett et al., 2006; Taipale et al., 2014), even at very low food quantities higher quality diets lead to better consumer growth (Boersma & Kreutzer, 2002; Schoo et al., 2012).

The ω3 and ω6 PUFAs are synthesized almost exclusively by primary producers, and certain phytoplankton classes, including cryptophytes, diatoms, dinoflagellates and chrysophytes, are rich sources of EPA, DHA and ARA (Taipale et al., 2013; Galloway & Winder, 2015). These bioactive $C_{20\text{-}22}$ PUFA (EPA, DHA and ARA) can also be produced from the shorter chain length homologues α-linolenic acid (ALA, 18:3ω3) and linoleic acid (LIN, 18:2ω6). The ability to elongate and desaturate C_{18} PUFA has been found in some freshwater zooplankton (Schlechtriem et al., 2006; Koussoroplis et al., 2014) and fish (Agaba et al., 2005), although

the efficiency of the process is considered to be low, and thus is not able to satisfy the optimal growth and reproduction requirements for the $C_{20\text{-}22}$ PUFA in the consumers (Bell et al., 2007). *Daphnia* can produce ARA by retroconversion of 22:5ω6, and they can similarly shorten C_{22} ω3 PUFAs to EPA (von Elert, 2002, Strandberg et al., 2014). Zooplankton are unable to synthesize PUFA *de novo* (Moreno et al., 1979), and, in general, endogenous fatty acid synthesis only accounts for < 2% of all fatty acids in *Daphnia* (Goulden & Place, 1990). Because $C_{20\text{-}22}$ PUFAs are essential for consumers and need to be obtained from the diet, food web dynamics can be heavily influenced by the fatty acid composition of the basal resources with PUFA-rich food sources leading to efficient transfer of energy through the food web and strong trophic coupling (Brett & Müller-Navarra, 1997; Müller-Navarra et al., 2004; Danielsdottir et al., 2007).

1.3 FATTY ACIDS AS BIOMARKERS

There are significant differences in the fatty acid composition among basal resources (phytoplankton, bacteria, tPOM) and also among consumer taxa (Persson & Vrede, 2006; Lau et al., 2012; Taipale et al., 2013; Galloway & Winder, 2015). The notion that many fatty acids are conservatively transferred from diet to consumer has led to the use of fatty acids as tracers in food web studies (reviewed by Dalsgaard et al., 2003).

Phytoplankton are the main synthesizers of PUFAs in aquatic ecosystems, and there are strong class-level multivariate differences in fatty acid composition of phytoplankton (Ahlgren et al., 1990, Taipale et al., 2013, Galloway & Winder, 2015). Cyanobacteria are rich in 16:0 and in some cases ALA, and do not synthesize $C_{20\text{-}22}$ PUFA or sterols (Ahlgren et al., 1990; Galloway & Winder, 2015; Volkman, 2003) which makes them a lower quality food source for zooplankton. Chlorophytes contain both ALA and LIN, but similar to cyanobacteria lack $C_{20\text{-}22}$ PUFA (Taipale et al., 2013). EPA and 16:1ω7 are characteristic of diatoms, while chrysophytes and cryptophytes are rich in a

variety of ω3 and ω6 PUFAs, including ALA, SDA, EPA, LIN and 22:5ω6. Dinoflagellates contain high proportions of EPA and DHA (Ahlgren et al., 1990; Galloway & Winder, 2015).

The fatty acid composition of heterotrophic bacteria is very different from phytoplankton. In general, bacteria lack PUFA, and are characterized by odd–chain SAFA, and *iso*– and *anteiso*–branched fatty acids (Actinobacteria) or SAFA and $C_{16–18}$ monounsaturated fatty acids (MUFA) (Proteobacteria) (Lechevalier and Lechevalier, 1988; Kaneda, 1991; Desvilettes et al., 1997). Leaves of terrestrial plants contain high levels of saturated fatty acids (SAFA), and are especially rich in very long chain SAFA (> C_{22}) (Taipale et al., 2015). They also have lower ω3:ω6 PUFA ratios than phytoplankton, and thus the ω3:ω6 ratio has been used as an index of the terrestrial contribution to lake food webs (Torres-Ruiz et al., 2007; Lau et al., 2012; Taipale et al., 2015). *Daphnia* survival, growth and reproduction are poor on diets of pure tPOM or bacteria, while mixes with >20% of phytoplankton can generally support zooplankton growth (Brett et al., 2009; Wenzel et al., 2012; Freese & Martin-Creuzburg, 2013; Taipale et al., 2014). Also, *Daphnia* growth efficiency is much lower on tPOM than on phytoplankton (5% versus ~40%, respectively) (Brett et al., 2009). Wenzel et al. (2012) attributed the poor quality of tPOM to low P and PUFA content. Bacteria also lack sterols, which are essential in sustaining *Daphnia* (Martin-Creuzburg & Von Elert, 2004). The quality of basal resources, especially in the case of sterol- and PUFA-poor tPOM and bacteria, can be upgraded by protozoans occupying the intermediate trophic steps in lake food webs (Klein-Breteler et al., 1999; Zhukova & Kharlamenko, 1999).

These compositional differences have allowed the use of fatty acids as chemotaxonomic markers to study seston composition. Earlier studies correlated individual fatty acids with the abundance of a certain phytoplankton group or species in the seston (Léveillé et al., 1997; Sushchik et al., 2004). Studies using multivariate techniques have demonstrated that at least a part of the variation in the fatty acid composition of mixed seston can be explained by the taxonomic composition of the plankton

community (e.g. phytoplankton, heterotrophic nanoflagellates and ciliates) (Kainz et al., 2009; Bec et al., 2010; Hessen & Leu, 2006). The whole fatty acid profiles of cultured algae have also been used to model the seston taxonomic composition (Dijkman & Kromkamp, 2006). The taxonomic composition of plankton can also be used in reconstructing the essential fatty acid content of seston in older time series in order to elucidate food web interactions (Hartwich et al., 2012; Galloway & Winder, 2015).

The prerequisite of using fatty acids as trophic markers is that the dietary signal is transferred to the consumer in a predictable way. Copepods are highly efficient in sequestering fatty acids from dietary algae, with up to 97% of all fatty acids removed during gut passage (Harvey et al., 1987). The fatty acid composition of *Daphnia* tracks the dietary signal with a lag phase of less than a week (Brett et al., 2006; Taipale et al., 2009, 2011). Similarly, the fatty acid composition of the freshwater copepod *Boeckella* was correlated with its diet, especially for SAFA and ω3 PUFA (Burns et al., 2011), although the turnover of the lipid pool in copepods is longer than in *Daphnia* (> 11 days) (Graeve et al., 1994, 2005). Ravet et al. (2010) found a connection between seston and zooplankton fatty acid composition in Lake Washington, especially for SAFA, ARA and EPA+DHA. However, several studies have reported that the fatty acid composition of field collected zooplankton is uncoupled from their potential diet (seston) (Persson & Vrede, 2006; Smyntek et al., 2008; Gutseit et al., 2007). In general, the concentrations of the essential PUFAs are higher in zooplankton than in seston, indicating that they are efficiently retained from the diet to meet physiological demands (Kainz et al., 2004; Smyntek et al., 2008; Mariash et al., 2011) which can partly explain why dietary signals are more difficult to identify in field collected consumers.

Detecting dietary influence in fish and other higher trophic level consumers gets more complicated compared to lower level consumers, because the turnover of their lipid pools is slower, which is especially true in adult fish. Lipids are added to the existing lipid pools when the fish grow and accumulate lipids thus increasing their total amount and diluting the existing lipids

rather than replacing them with new, dietary lipids (Castledine & Buckley, 1980; Jobling, 2004; Benedito-Palos et al., 2009). In the storage lipids of fast-growing herring larvae, the PUFA 18:4ω3 peaked only 23 days after the initial peak in phytoplankton, while zooplankton incorporated this fatty acid at a faster rate (Fraser et al., 1989). Cod larvae exhibited a ratio of 16:1ω7 : 16:0 indicative of diatoms after only 13 days of feeding on copepods that were reared on diatoms (St. John & Lund, 1996). Despite the lower fatty acid turnover rates, the diets of adult fish, seals, seabirds and polar bears have been successfully elucidated using quantitative fatty acid signature analysis (QFASA, Iverson et al., 2004, 2007; Thiemann et al., 2007; Tucker et al., 2009; Budge et al., 2012).

1.4 AIMS OF THE STUDY

The goal of the study was to explore the transfer of fatty acids from phytoplankton to planktivorous fish in the pelagic food webs of boreal lakes. More specifically, I was interested in the role of copepods in the fatty acid dynamics of lakes, as they have rarely been studied at high taxonomic resolution in freshwater systems. The specific objectives were

1. to assess if phytoplankton community structure can be reliably derived from the fatty acid composition of seston (I)
2. to study the magnitude of the differences in fatty acid composition among zooplankton taxa (II)
3. to examine the within-taxa seasonal and spatial variation in zooplankton fatty acid composition, and to link it to their life-cycle strategies, diet and environmental conditions (II, III, IV)
4. to explore fatty acid transfer through aquatic food webs from seston to fish and to find out if essential PUFAs are retained and biomagnified relative to other fatty acids. (V)

2 Material and methods

2.1 FIELD SAMPLING AND SAMPLE PREPARATION

The transfer of fatty acids in the food web, as well as seasonal and spatial differences in fatty acids of seston, zooplankton and fish were investigated using extensive field sampling. Most of the samples for the analysis of fatty acids and stable isotopes were collected in the Vuoksi watercourse in Eastern Finland (Fig 2.). Seven of the locations in large lakes were sampled three times: in the spring (19-27 May 2011), summer (1-4 August 2011), and autumn (26-28 September 2011), and one location (Karjalan Pyhäjärvi) was sampled once (6 August 2012). Additional sampling for the copepod *Limnocalanus macrurus* was done in two of these locations (Suva 399 and Pyhä 5) in the winter (November, March) (III), and *Eudiaptomus* spp. samples were collected once from 19 additional lakes in boreal and subarctic regions of Finland to provide a better spatial gradient (IV).

Seston (<50 μm) was filtered on precombusted GF/F filters (Whatman), and immediately dissolved in a 2:1 chloroform:methanol solution (I). To obtain seston samples for stable isotope analyses we pumped water onboard and filtered it through 50 μm net to exclude macrozooplankton before concentrating it with a tangential filtration (0.2 μm) device (Millipore).

Zooplankton was collected with vertical tows of a plankton net (50-200 μm) through the whole water column or with horizontal tows of Hydrobios Multi Plankton Sampler (mesh size 100 μm) above and below the thermocline (II-V). Zooplankton samples were frozen in the field, and later briefly thawed in the laboratory and sorted in most cases to the genus level under a dissecting microscope. Nine different taxa were analyzed: the calanoid copepods *Limnocalanus macrurus*, *Heterocope* spp., *Eurytemora lacustris*, and *Eudiaptomus* spp.; cyclopoid copepods

(not identified to genus); filter-feeding cladocerans (not identified to genus), the predatory cladoceran *Bythotrepes longimanus*; dipteran larvae *Chaoborus* spp.; and the glacial relict macrocrustacean *Mysis relicta*. Fish were caught by gillnetting or trawling, or obtained from local commercial fishermen (V). Dorsal muscle of the fish was dissected and frozen for fatty acid and stable isotope analyses.

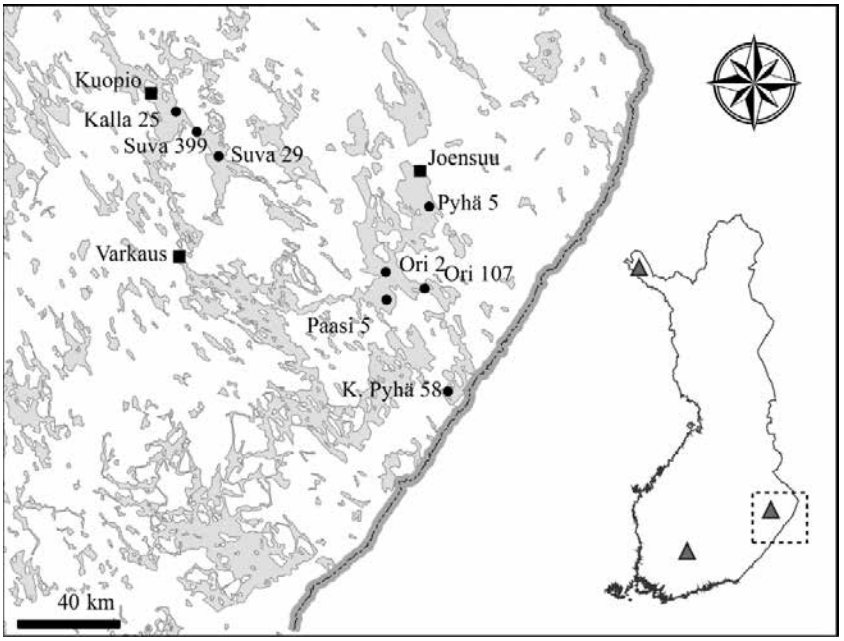

Figure 2. *Detailed map of the major towns nearby (black squares) and the locations (black circles) that were sampled for fatty acid analysis of the whole food web from seston to fish in three seasons (I-III, V). Grey triangles indicate approximate locations of lakes (n = 25) that were sampled for fatty acids of Eudiaptomus (IV). Kalla = Kallavesi, K. Pyhä = Karjalan Pyhäjärvi, Ori = Orivesi, Paasi = Paasivesi, Pyhä = Pyhäselkä, Suva = Suvasvesi. The map contains data from the National Land Survey of Finland Topographic Database 06/2012. (http://www.maanmittauslaitos.fi/en/NLS_open_data_licence_version1_20120501)*

Water chemistry parameters, including chlorophyll *a*, total nitrogen, total phosphorus, and water color, were analyzed using standard methods (international standards ISO 10260:1992, ISO 11905-1:1998, ISO 6878:2004, ISO 7887:2011). Dissolved organic carbon (< 0.22 μm) was analyzed with a Multi NC Instrument

(Analytik Jena). Samples for phytoplankton community composition enumeration were preserved with acid Lugol's solution. Subsamples of 25-50 mL were settled and all the cells were counted in size-classes from 25 fields with magnifications of 500x (small cells) and 250x (large cells) with an inverted microscope (Leitz Fluovert FS) (I, II).

2.2 LIPID ANALYSIS

Lipids were extracted twice from lyophilized zooplankton and fish tissue (0.2 – 2.0 mg DW) and from the seston filters with a 2:1 chloroform:methanol (by vol) solution and the chloroform phases that contained the lipids were pooled (Folch et al., 1957). For analysis of total lipids (II-V), the whole extract was subsequently transmethylated. Solid phase extraction with silica cartridges (500 mg, Agilent) was used to separate phospholipids in the seston samples (I).

Eleven *Limnocalanus* lipid samples were subjected to additional analyzes, including wax esters and sterols (III). Wax esters were first dissolved into ethyl acetate and run intact with a gas chromatograph mass spectrometer (Agilent 6890N and 5973) equipped with a programmable temperature vaporizing inlet (PTV, Atas Optic 4). Subsequently the remaining lipid extract was used to separate wax esters with thin layer chromatography on silica-coated plates with a mobile phase of hexane:diethyl ether:acetic acid (90:10:1, by vol). Trimethylsilyl ethers of sterols were run using a gas chromatograph mass spectrometer (Shimadzu Ultra) at the University of Jyväskylä.

Fatty acid methyl esters (FAMEs) of total lipids, phospholipids or wax esters were produced with acid-catalyzed transmethylation (Christie, 1993), and analyzed with a gas chromatograph equipped with mass selective detector (Agilent 6890N and 5973N). The column was Agilent DB–23 (30 m x 0.25 mm x 0.15 μm) and helium was used as the carrier gas with an average velocity of 34 cm s^{-1}. For most of the samples, the following temperature program was used: oven temperature was

held at 60°C for 1.5 min^{-1}, then increased 10°C min^{-1} to 100°C, followed by 2°C min^{-1} to 140°C, and 1°C min^{-1} to 180°C, and finally 2°C min^{-1} to 210°C, and then held for six minutes. Additional details of the laboratory methods can be found in the original publications.

2.3 STABLE ISOTOPE ANALYSIS

Stable isotopes of nitrogen (V) were analyzed from freeze-dried seston, zooplankton and fish samples with a Carlo-Erba Flash 1112 series Element Analyzer connected to a Thermo Finnigan Delta Plus Advantage Isotope-ratio mass-spectrometer (University of Jyväskylä).

2.4 STATISTICAL ANALYSIS AND MODELLING

All the multivariate statistics on fatty acid composition data were run on Euclidean distances. Non-metric multidimensional scaling (NMS) was used for visualizing the patterns in fatty acid composition among taxa, seasons and lakes (I-V). NMS is an iterative ordination method that reduces the dimensions of the data while preserving the rank order of distances between samples. The stress value represents how well the final ordination can present the variation in the original data, with values ca. < 0.15 indicating a reliable ordination. Pearson correlations with the produced NMS axes were used to identify the fatty acids that influenced the ordination the most.

Permutational multivariate analysis of variance (PERMANOVA, Anderson et al., 2008) was used to test for differences in fatty acid composition among taxa (II, V), seasons (I-III) and lakes (I, IV). Permutational multivariate analysis of variance was run with type III sums of squares and unrestricted permutation of raw data. The spatial differences in fatty acid composition of zooplankton were investigated using distance-based linear modelling (Anderson et al., 2008) (II, IV). Distance-

based linear modelling is based on redundancy analysis, and it is used for detecting the linear effect of multiple explanatory variables on multivariate response data.

Fatty acid source tracking algorithm in R (FASTAR) is a Bayesian mixing model that has been adapted from the stable isotope mixing model MixSIR (Moore and Semmens, 2008; Galloway et al., 2015). FASTAR was used for inferring the phytoplankton community composition from seston fatty acids (I). In addition to the seston fatty acid profiles, the input data consisted of fatty acid composition (mean and SD) of monocultures of different algal classes (the reference library), which FASTAR uses to calculate the potential proportions of algal classes contributing to the seston samples collected in field.

3 Results and discussion

3.1 FATTY ACIDS AT THE BASE OF THE FOOD WEB – SESTON

The POM suspended in water (seston) comprises both living and non-living components, and includes a mix of various phytoplankton groups, heterotrophic flagellates, ciliates, bacteria, and autochthonous and terrestrial detritus with their contributions varying both seasonally and spatially. The biochemical quality of POM in lakes is seasonally more variable than the elemental composition (C, N, P), and lipids generally form a larger fraction of POM than carbohydrates or protein (Kreeger et al., 1997). Fatty acid composition differs significantly among phytoplankton classes, which indicates that fatty acids could be a good tool for inferring community composition of mixed seston samples (Taipale et al., 2013; Galloway & Winder, 2015).

The current study used a Bayesian fatty acid-based mixing model called FASTAR (Galloway et al., 2015) to elucidate the phytoplankton community composition in moderately-humic large lakes, and compared the results with microscopy (I). The results of FASTAR and microscopic counts were highly correlated (r = 0.596, p < 0.001), and both methods identified cryptophytes and diatoms as the main phytoplankton groups in the large lakes (Fig. 3), together comprising on average >50% of total carbon biomass. The dominance of these taxa is consistent with the physico-chemical characteristics of the studied lakes. In general, diatoms have a competitive advantage in turbulent conditions that are common in wind-exposed large lakes (Huisman et al., 2004). Cryptophytes, on the other hand, are typically abundant in lakes with a moderate to high humic content (Lepistö & Rosenström, 1998).

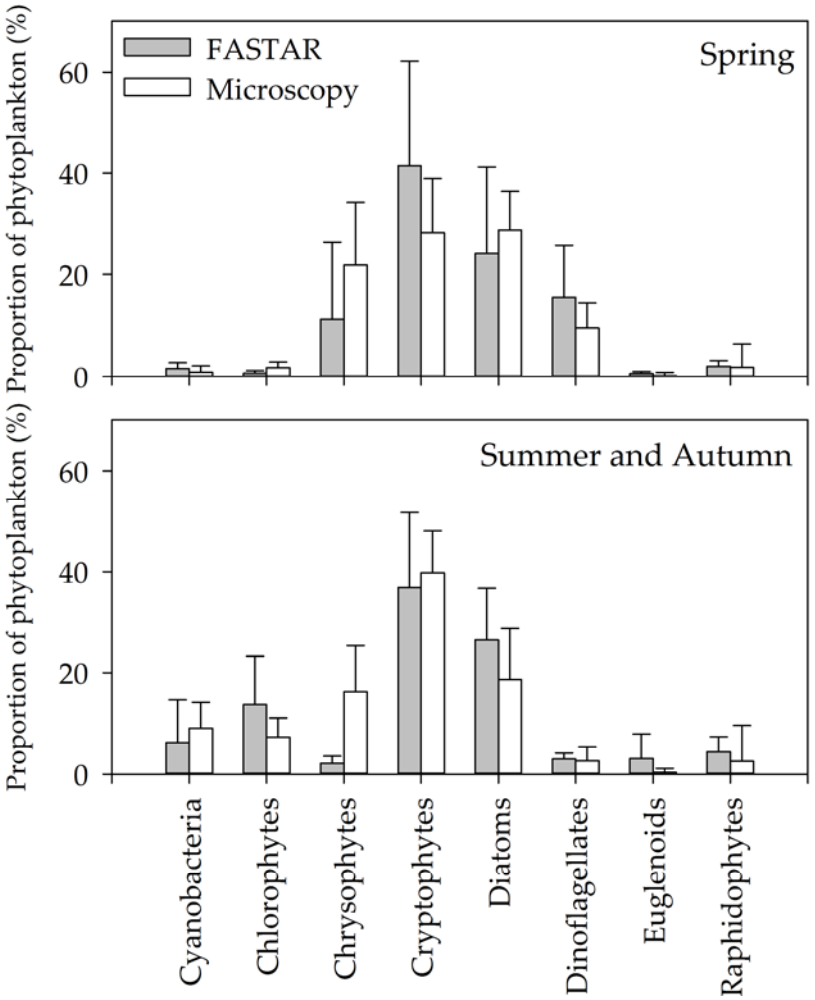

Figure 3. *Phytoplankton community composition (mean ± SD) in the spring (n = 6), and in the summer and autumn (n = 14) in the large, moderately humic lakes. Estimates were made by microscopy and FASTAR modelling based on seston PLFAs (I).*

The high correlation between estimates derived with microscopy and modelling suggests that the compositional differences in the phytoplankton phospholipids are sufficiently large to accurately identify the contribution of phytoplankton classes to mixed seston samples. Only few fatty acids are truly taxon-specific (e.g. certain C_{16} PUFAs for diatoms, Dijkman & Kromkamp, 2006), but the multivariate approach using the whole fatty acid composition can provide an accurate separation of phytoplankton classes (Taipale et al., 2013; Galloway & Winder,

2015). Environmental conditions (light, nutrients, temperature) can also modify the fatty acid composition of phytoplankton, but a recent meta-analysis showed that the proportion of variation related to phylogeny is 3-4 times higher (Galloway & Winder, 2015).

Although FASTAR performed well in most cases, there was a discrepancy between the two methods in estimating the abundance of chrysophytes: FASTAR did not indicate chrysophytes as a major phytoplankton group, although microscopy found them to form ~18% of the total carbon biomass (Fig. 3). This difference could be because the reference library was inadequate for chrysophytes, with fatty acid data for only three taxa available, and a major genus (*Pseudopedinella*) found in lake samples was unfortunately not represented in the FASTAR model. *Pseudopedinella* was not available in culture collections, and published fatty acid data for freshwater strains were not found. Additionally, chrysophytes and cryptophytes have a similar fatty acid composition (Taipale et al., 2013) and it is possible that FASTAR had difficulty separating their contribution to the seston fatty acid pool.

Heterotrophic flagellates and ciliates formed 7-11% of the carbon biomass according to microscopic counts, but were not included in the FASTAR analysis because of the lack of appropriate fatty acid data for reference library. Heterotrophic nanoflagellates and ciliates can be an important PUFA source in seston (Bec et al., 2010; Hartwich et al., 2012). In general, the fatty acid composition of heterotrophic flagellates and ciliates is highly variable, and may be related to their diet (Véra et al., 2001; Boëchat & Adrian, 2005). The proportion of bacterial fatty acids (branched and odd-chain SAFA) was always low in the seston phospholipids.

Both methods identified that the phytoplankton community in the spring was different from summer and fall, with a higher abundance of dinoflagellates in the spring and chlorophytes and cyanobacteria in the summer and fall (I, Fig. 3). Cryptophytes, diatoms and dinoflagellates have been recognized as key taxa in producing essential fatty acids at the base of aquatic food webs

(Galloway & Winder, 2015). Although there was generally a high abundance of PUFAs in the seston throughout the sampling season (>40%), this dominance of high quality phytoplankton in spring is reflected in the fatty acid composition of seston, with ~25% of the essential $C_{20\text{-}22}$ PUFAs. The proportion of $C_{20\text{-}22}$ PUFAs declined to 9-13% in summer and fall, corresponding to an increase in cyanobacteria and chlorophytes in the phytoplankton community. The biomass of phytoplankton was higher in the spring and summer than in fall, which indicates that both the quantity and quality of seston available for zooplankton consumption were high in the spring. Similar to our study, Müller-Navarra & Lampert (1996) found the food quantity and quality for zooplankton to be high in the spring. A spring diatom bloom that is a common feature of phytoplankton dynamics in many lakes (Reynolds 2006) also offers a pulse of high quality food for benthic consumers (Goedkoop et al., 2000).

With the help of the program CHEMTAX, HPLC-derived pigments have been widely used as chemotaxonomic markers for phytoplankton groups especially in the marine environment (Mackey et al., 1996), although there are difficulties in separating certain phytoplankton groups based on pigments alone (Lewitus et al., 2005). A previous study demonstrated that phytoplankton community composition derived from phospholipid fatty acids matches with results from pigment analyses using CHEMTAX (Dijkman & Kromkamp, 2006). Furthermore, the results from the current study indicate that fatty acid mixing models are promising tools to track phytoplankton dynamics at the group level. Compared to pigments, the advantage of using fatty acids is that the greater number of biomarkers can result in more robust estimates of phytoplankton community structure (Dijkman & Kromkamp, 2006).

3.2 FATTY ACID COMPOSITION OF ZOOPLANKTON

Large part (62%) of the variation in zooplankton fatty acid composition could be explained by species identity (II, Fig. 4),

indicating that fatty acid composition is largely under metabolic regulation that is ultimately controlled by evolutionary history. Copepods had higher proportions of DHA, and PUFAs in general, than cladocerans, although cladocerans were richer in ARA than copepods. This pattern of copepods being rich in DHA while cladocerans containing mainly EPA and ARA is well established in the literature (Persson & Vrede, 2006; Smyntek et al., 2008; Burns et al., 2011), and it has been hypothesized to exist because copepods have more highly developed nervous system than cladocerans (Persson & Vrede, 2006), and copepods require more DHA when inhabiting cold waters of hypolimnion or during overwintering (Farkas, 1979; Smyntek et al., 2008). The high demand of EPA in cladocerans has been linked to short generation time and high reproductive rate that necessitate intensive eicosanoid synthesis (Smyntek et al., 2008).

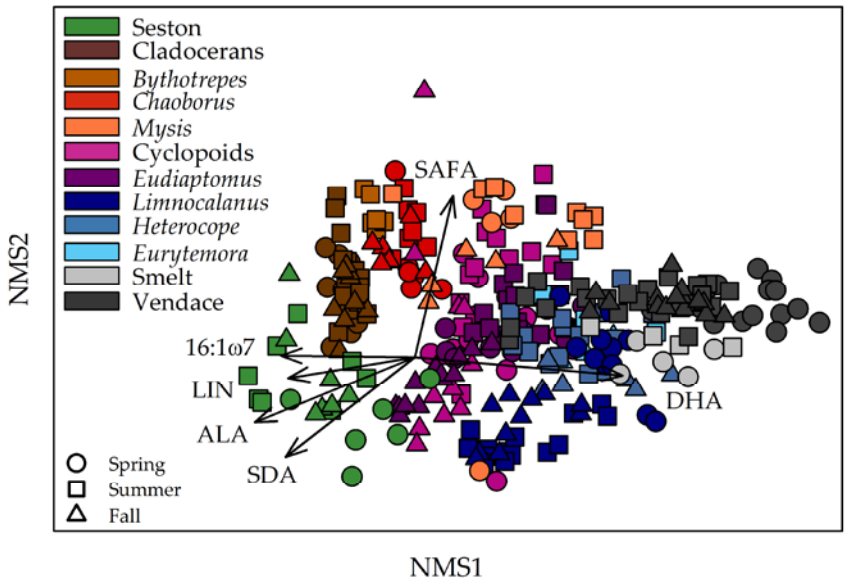

Figure 4. *Non-metric multidimensional scaling (NMS) ordination of the fatty acid composition (33 FA) of seston (n = 21), zooplankton (n = 227) and fish (n = 80) in large boreal lakes. Fatty acids that correlate strongly (Pearson's r > 0.6) with either of the axis are shown as vectors. Stress for the 2-dimensional solution was 0.13 (II, V).*

Previous studies have reported that predatory copepods and cladocerans are richer in EPA, DHA and ARA than herbivorous

taxa (Persson & Vrede, 2006; Smyntek et al., 2008; Ravet et al., 2010). In the current study the larger copepods *Limnocalanus* and *Heterocope* had higher proportions of EPA and DHA than the smaller taxa *Eudiaptomus* and cyclopoids, although studies on their feeding ecology indicate that all of these taxa can be considered omnivores (II). Similarly, the filter-feeding cladocerans had lower proportions of EPA and ARA than the predatory *Bythotrepes*. The glacial relict macrocrustacean *Mysis* was also rich in EPA and DHA, and together with the dipteran larvae *Chaoborus,* it had conspicuously high levels of 18:1ω9 which has previously been found in many benthic macrocrustaceans (Lau et al., 2012).

There was pronounced seasonality in the fatty acid content and composition in two of the copepod species, *Eudiaptomus* and *Limnocalanus* (II-III). Both of them accumulated lipids towards fall, and *Limnocalanus* attained maximum lipid reserves already in August. These calanoid copepods overwinter in an active stage, and the accumulation of lipid reserves is likely a strategy to cope with food scarcity in winter. A laboratory experiment demonstrated that *Eudiaptomus* depletes triacylglycerol reserves rapidly during food shortage, which indicates that *Eudiaptomus* is not able to withstand prolonged periods of starvation (Koussoroplis et al., 2014). The proportions of ALA and SDA increased in these copepods with increasing lipid reserves, which is most likely connected to the availability of these C_{18} PUFAs in the seston throughout the open water season (~30 % of total, I). However, also SAFA and MUFA were consistently available in the seston but were not accumulated in the copepods, which suggests that the formation of lipid reserves is under physiological control, and certain PUFAs are preferentially retained while SAFA and MUFA are either used for fulfilling the needs of basic metabolism, preferentially excreted or simply not efficiently assimilated (Graeve et al., 2005; Mariash et al., 2011). *Daphnia* incorporated the SAFA 20:0 less efficiently than EPA or ARA when fed phytoplankton enriched in these fatty acids (Becker & Boersma, 2005). The lipid reserves of many marine copepods, however, contain high abundances of $C_{20\text{-}22}$ MUFAs

which are a result of endogenous synthesis (Graeve et al., 2005; Lee et al., 2006).

The lipid reserves in the glacial-relict calanoid copepod *Limnocalanus* consist of wax esters similar to marine copepods instead of triacylglycerols which are more common in freshwater taxa (Cavaletto et al., 1989). The wax esters of *Limnocalanus* contained mostly saturated fatty alcohols, and mono- and polyunsaturated fatty acids (III), which suggests that dietary SAFAs are transformed to alcohols before incorporating them to wax esters with unsaturated fatty acids (Lee et al., 2006). The wax ester reserves of *Limnocalanus* were mobilized slowly during the winter, and likely were directed to reproduction in the early spring. Similar to some marine copepods, the polyunsaturated wax esters in *Limnocalanus* were metabolized first in the winter (Lee et al., 1970), and also *Eudiaptomus* in a laboratory study used PUFAs more quickly than SAFAs or MUFAs during fasting (Koussoroplis et al., 2014). In contrast, the PUFAs ARA and EPA were highly conserved in *Daphnia* during starvation (Schlechtriem et al., 2006). *Daphnia* do not accumulate storage lipids in a similar way to these copepods, and thus a larger fraction of their lipids are bound in membranes that need to remain functional even during starvation. Compositional changes in the wax esters of *Limnocalanus* during the winter indicated that the copepods stayed active and fed at least sporadically, with the increase in bacteria-related fatty acids suggesting that they most likely fed on heterotrophic sources (III). In contrast to *Limnocalanus*, Arctic marine copepods spend the winter in diapause after having very rapidly accumulated wax esters during the peak of primary production in the spring (Lee et al., 2006). The sterol composition of *Limnocalanus* was dominated by cholesterol (III), which is consistent with studies on other copepods (Mühlebach et al., 1999; van der Meeren et al., 2008).

Throughout the year, *Limnocalanus* had very high proportions of PUFA (up to > 70%), and it was the only taxa that had very long chain PUFAs (VLC-PUFAs, $C_{24\text{-}26}$), which generally are rare in aquatic ecosystem (but see Řezanka, 1989; Řezanka & Dembitsky,

1999; Mansour et al., 1999). VLC-PUFAs were present in August (7.8 ± 4.3% of total fatty acids) and September (2.7 ± 2.1%), and were found both in the lake and the Baltic Sea samples of *Limnocalanus* (III). The most abundant VLC-PUFAs were always 24:4ω3 and 24:5ω3, and they contribute to separation of *Limnocalanus* from other zooplankton taxa in the NMS plot (fig. 4). VLC-PUFAs were not present in the potential food sources of *Limnocalanus* (seston and other zooplankton), and thus it is likely that the copepods synthesize these fatty acids from shorter chain PUFAs. VLC-PUFAs were found in the wax esters, which suggests that their function may be to maximize the storage lipids in *Limnocalanus*. We found trace levels of VLC-PUFA in planktivorous fish (vendace) in these same lake basins, and these fatty acids have been previously detected in Baltic herring and in seals from the study lakes and the Baltic (Linko & Karinkanta, 1970; Käkelä et al., 1995), which indicates that VLC-PUFAs are transferred up the food chain. The VLC-PUFAs could provide a useful indicator of the contribution of *Limnocalanus* in the food web, if their metabolism in fish feeding on *Limnocalanus* was resolved in controlled feeding trials.

In addition to the substantial taxonomic and seasonal variation in zooplankton fatty acid composition, there were also spatial differences. The most obvious differences were seen when comparing samples of *Limnocalanus* from the lakes and from the brackish-water Baltic Sea: *Limnocalanus* from the Baltic Sea had three times the proportion of 18:1ω9, and lower proportions of EPA and DHA than *Limnocalanus* from the large lakes (III). Similar to *Limnocalanus*, *Pseudocalanus* in the Baltic had high abundances of 18:1ω9, which was attributed to the importance of ciliates in the diet of the copepods (Peters et al., 2006).

More subtle spatial changes in the fatty acid composition were revealed in five zooplankton taxa in relation to water chemistry in the large lakes (II). In the larger dataset of 25 lakes the fatty acid composition of the copepod *Eudiaptomus* was also connected to water chemistry, and additionally to lake size (Fig. 4, IV). In particular, the spatial differences in water chemistry (nutrients, chlorophyll *a*, and DOC) were connected to the ratio of ω3 and

ω6 PUFAs in the zooplankton of the large and clearwater lakes but not in the small and humic ones (Fig. 4, II, IV). The ω3:ω6 ratio in zooplankton increased with increasing DOC, nutrients and chlorophyll *a*. Previously, the ω3:ω6 ratio in the benthic isopod *Asellus aquaticus* has been found to increase with increasing lake trophic state, which has been suggested to be connected to dominance of diatoms in benthic communities of eutrophic lakes (Lau et al., 2013).

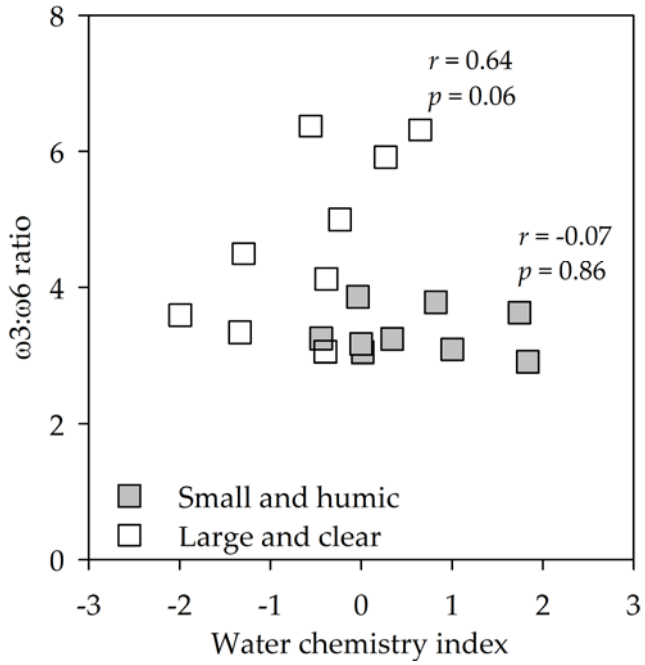

Figure 5. *The ratio of ω3 and ω6 PUFAs in Eudiaptomus in relation to water chemistry index (Pearson's r). Water chemistry index was constructed with principal component analysis from the correlated water chemistry parameters, and when the index increases, nutrient concentrations, DOC, chlorophyll a, and water color increase. N = 9 for both lake types (IV).*

The spatial differences in zooplankton fatty acid composition could be related to, for example, variation in diet and/or water temperature. Laboratory studies have demonstrated that zooplankton fatty acid composition is highly influenced by their diet (Brett et al., 2006; Caramujo et al., 2008; Bourdier & Amblard, 1989; Burns et al. 2011). Only a few previous field studies have

demonstrated a connection between the fatty acid composition of seston and zooplankton (Ravet et al. 2010, Burns et al. 2011), while other field studies observed no connection between the two (Persson & Vrede, 2006; Smyntek et al., 2008). Fraser et al. (1989) showed that seston lipids not only influence the fatty acid composition of zooplankton, but also the lipids of zooplanktivorous fish larvae, indicating that lipids produced at the base of the food web can have an impact on higher trophic levels.

In the data set comprising data from large lakes, the fatty acid composition of five zooplankton taxa seemed to be influenced indirectly by DOC with lakes that had higher DOC concentrations having a lower proportion of chlorophytes and higher proportion of diatoms than lakes with low DOC (II). In contrast, the abundance of bacterial fatty acids (*i-* and *ai-*branched fatty acids, and odd-chain SAFA) or long-chain SAFAs indicative of terrestrial vegetation were not connected to lake DOC concentration (paper II), demonstrating that the contribution of allochthonous resources to zooplankton did not change along the DOC gradient. However, long-chain SAFAs are not efficiently retained in *Daphnia* (Brett et al., 2009; Taipale et al., 2015) or in *Eudiaptomus* (Sami J. Taipale, unpublished results), and thus they seem to be unsuitable biomarkers for quantifying the contribution of tPOM in aquatic consumers. Allochthonous resources in lakes have low proportions of PUFAs, and thus are less important than phytoplankton in the PUFA trajectories of lake food webs. Furthermore, high contribution of tOM of low biochemical quality results in poor production of zooplankton (Brett et al., 2009; Kelly et al., 2014). The cladocerans in the studied large boreal lakes were shown to rely mostly on autochthonous sources in a previous study (Galloway et al., 2014).

In the 25 lake data set, phytoplankton data were not available for all lakes where *Eudiaptomus* was sampled, and thus the fatty acid composition of *Eudiaptomus* could not be compared to phytoplankton community structure. However, changes in phytoplankton community would be likely along the broad

water chemistry gradient and differences in lake sizes that the lakes represent. Gutseit et al. (2007) found seston in humic lakes to have higher EPA content than seston in clearwater lakes, and attributed this to differences in the phytoplankton assemblages among the lake types, and more specifically to the abundance of the raphidophyte *Gonyostomum semen* in the humic lakes. Persson et al. (2007) observed that seston EPA concentration had a unimodal connection to lake trophic state, with highest EPA concentration at ~10 μg P L^{-1}, and concluded that based on the modeling results *Daphnia* growth would likely be food quantity limited at low P concentrations (< 4 μg P L^{-1}) and EPA limited at high P concentrations. Thus, moderate nutrient additions to an oligotrophic lake may boost primary production and consumer growth if the phytoplankton community does not shift to species of low nutritional value (Rydin et al., 2008).

Fluctuations in environmental temperature can prove fatal for poikilothermic animals, such as crustaceans, partly because temperature affects the fundamental structure and functioning of cell membranes, and thus adapting to temperature changes is crucial to the survival of organisms (Pruitt, 1990). Farkas (1979) found that copepods had more DHA in their phospholipids in colder temperatures and attributed this to maintaining membrane fluidity in the cold (homeoviscous adaptation). In addition to membrane lipids, the storage lipids can become more unsaturated during cold acclimation (Saether et al., 1986; van Dooremalen & Ellers, 2010). The freshwater cladoceran *Daphnia* had higher proportions of EPA and MUFA in its total lipids when grown in 11°C compared to 22°C (Schlechtriem et al., 2006), and *Daphnia* also have a higher requirement for EPA in colder temperatures to be able to reach optimum growth rates and to reproduce successfully (Sperfeld & Wacker, 2011, 2012). Similarly, several studies have found a connection between water temperature and the abundance of PUFA or SAFA, for instance in ciliates (Martin et al., 1976), crustaceans (Pruitt, 1990), and fish (Farkas et al., 2001), and in the current study, SAFA was more abundant in cyclopoids and *Eudiaptomus* in the summer when the water temperatures were close to 20°C than in the spring or fall

when the temperatures were ~9 °C and ~13 °C (II). *Eudiaptomus* in subarctic lakes had a significantly higher proportion of DHA than in boreal lakes, although the average temperatures among the lakes were only slightly different during the sampling in autumn (10.0 ± 3.0°C versus 13.5 ± 1.4°C respectively) (IV). Furthermore, there were no clear differences in the DHA proportion of *Eudiaptomus* among the subarctic lakes, although their water temperature ranged from 6.5°C to 13.7°C. Interestingly, many Arctic/Antarctic marine copepods that regularly endure very cold temperatures accumulate $C_{20\text{-}22}$ MUFAs instead of PUFAs, and the proportion of PUFA in their lipids can be as low as 11% (Kattner et al., 2003; Lee et al., 2006).

A glycerophospholipid molecule has two fatty acids and one phosphorus-containing polar headgroup attached to a glycerol backbone. One of the fatty acids generally is 16:0 while the other one is a PUFA, most commonly DHA, EPA, or ARA. A phospholipid molecule with only one double-bond has a gel to liquid crystalline phase transition temperature (e.g. melting point) of ~0°C, and with two double-bonds ~-15°C, whereas the further addition of double-bonds does not significantly decrease the melting point, or can even increase it (Stillwell & Wassal, 2003). The low melting points achieved with only two double-bonds indicate that there must be other reasons besides maintaining membrane fluidity in using PUFAs with four to six double-bonds that are easily oxidized. Furthermore, although studies show that the abundance of DHA in cell membranes is highly affected by temperature (e.g. Farkas, 1979), EPA (as a free fatty acid) has a lower melting point (-54°C) than DHA (-44°C) (Whitcutt & Sutton, 1956; Whitcutt, 1957), which would potentially make EPA better in maintaining the fluidity of membranes in cold temperatures. The role of DHA in cell membranes is not yet thoroughly understood, but in addition to affecting fluidity, DHA influences the fusion, permeability and elastic compressibility of cell membranes, and the activity of membrane bound proteins (Stillwell & Wassal, 2003). Other mechanisms by which membranes adapt to cold include changing the composition of the phospholipid headgroups, the

amount of cholesterol in the membrane and the chain-length of the fatty acids (Pruitt 1990, Ohtsu et al., 1998; Stillwell & Wassal, 2003). Koussoroplis et al. (2014) suggested that copepods may change the composition of their cell membranes (in a similar way as during cold acclimation) to reduce their metabolic rate to prolong survival during food shortages.

The fatty acid composition of zooplankton as food for fish can have important consequences to fish survival, growth and reproduction (Sargent et al., 1999; Paulsen et al., 2014). A bioenergetics model predicted that the growth of sockey salmon in Lake Washington may be suppressed by the low availability of DHA in the zooplankton prey (Ballantyne et al., 2003). The large variation in zooplankton fatty acid composition across taxonomic groups, seasons and environmental conditions in the present studies suggest that fish can experience considerable differences in their food quality (II-IV). Climate change induced changes in temperature and precipitation patterns are projected to lead to notable changes in water chemistry of lakes in the boreal region, more specifically to increased runoff of DOC and nutrients (Lepistö et al., 2008; Larsen et al., 2011). While increased water temperatures and nutrient loading may promote the growth of cyanobacteria (Elliot, 2012; Anneville et al., 2015), the influence of DOC on phytoplankton assemblages may be more complicated. Decreased light penetration can lead to overall suppressed primary production (Thrane et al., 2014; Seekell et al. 2015), thus reducing food quantity to zooplankton, but may also result in dominance of PUFA-rich phytoplankton species that are highly nutritious for zooplankton, which can result in increased food web efficiency and thus enhance fish production (Brett & Müller-Navarra, 1997; Müller-Navarra et al., 2004; Danielsdottir et al., 2007; Gutseit et al., 2007).

3.3 TRANSFER OF FATTY ACIDS TO FISH

Several laboratory and field studies in the marine environment have demonstrated that the effects of food quality on the basal

level of ecosystems can cascade up the food chain to fish (Fraser et al., 1989; St John & Lund, 1996; Pederson et al., 1999; St. John et al., 2001; Schoo et al., 2012). Corresponding studies in freshwater systems that span from seston to fish are rare (but see Kainz et al., 2004; Ravet et al., 2010; Lau et al., 2011), and there is a growing need to understand how essential fatty acids are transferred through the food web in lakes. Furthermore, large lakes are socio-economically important for recreational and navigation purposes, and they harbor productive fisheries (Krantzberg & de Boer, 2006; Nõges et al., 2008). Lake size influences several physico-chemical and biological factors, including mixing patterns, water chemistry, phytoplankton and zooplankton community composition (Lepistö & Rosentröm, 1998; Nõges et al., 2008). Thus, there might be differences also in the trophic transfer of fatty acids between large lakes and the more often studied small and moderately sized lakes (e.g. Kainz et al., 2004; Ravet et al., 2010; Lau et al., 2012). In the current study seston, zooplankton and planktivorous fish were sampled to investigate which fatty acids were retained and enriched in the food web (V). The stable isotope ratio of nitrogen ($\delta^{15}N$) was used to represent the trophic positions of the organisms (McCutchan et al., 2003).

DHA was highly enriched up the food web with low levels in seston (~4% of total PLFAs) and high levels in the planktivorous fish vendace (*Coregonus albula*) and smelt (*Osmerus eperlanus*) (~32% of total fatty acids) (Fig. 6). The DHA levels in the studied freshwater fish are comparable to those of marine fish which have ~10-30% of DHA (Budge et al., 2002), which demonstrates that freshwater fish can be a very good source of essential ω3 fatty acids in human diets. DHA has been highly retained also in rainbow trout (Kainz et al., 2004) and juvenile sockeye salmon (Ravet et al., 2010). The efficient accumulation of DHA is most likely related to its important role in fish physiology (Sargent et al., 1999). DHA is particularly crucial in the early development of fish; the survival and growth of marine fish larvae has been linked to tissue DHA levels, which were influenced by their diet (Cutts et al., 2006; Paulsen et al., 2014).

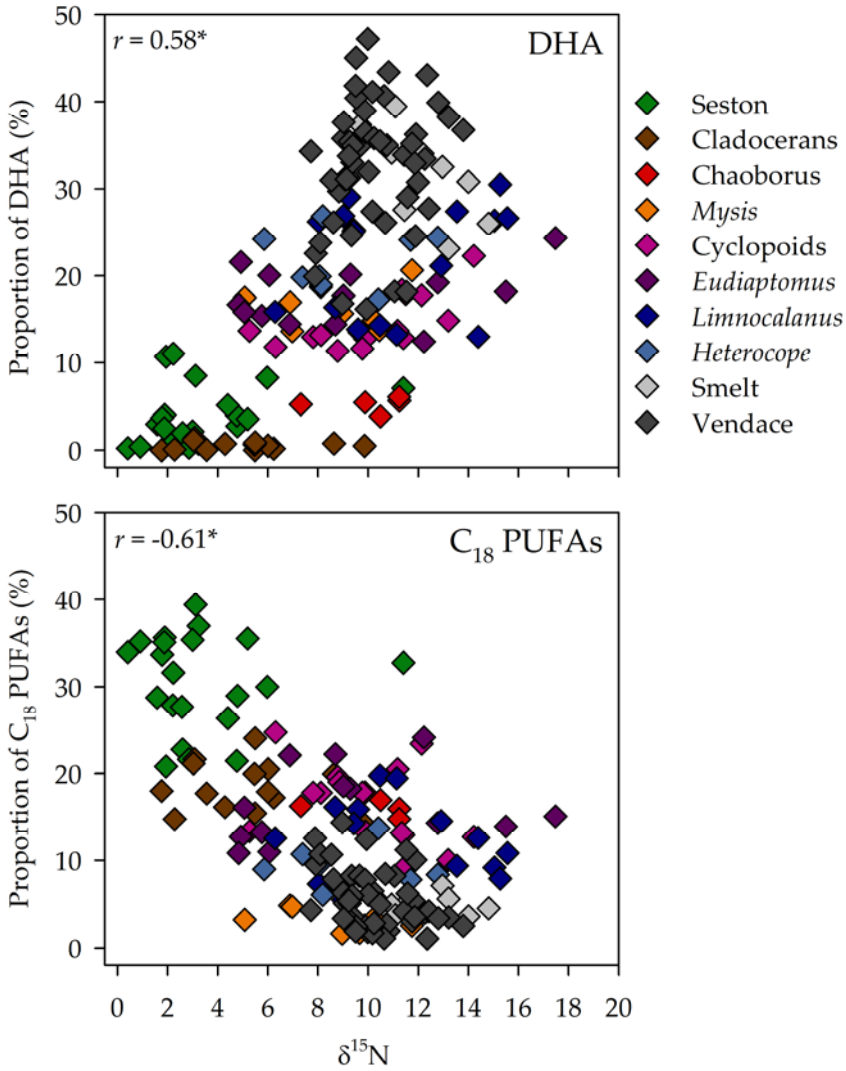

Figure 6. *Trophic transfer of DHA and C_{18} PUFAs in the food webs of large boreal lakes. Significant correlations (Pearson's r) denoted with *. N = 181.*

In addition to DHA, the proportion of EPA increased with increasing $\delta^{15}N$ in the current study, but the magnitude was less striking than with DHA. The highest EPA levels were found in *Chaoborus*, *Mysis* and *Heterocope* (~18%) with somewhat lower values in fish (9% and 15%, for vendace and smelt, respectively). In contrast to EPA and DHA, the proportions of the C_{18} PUFAs ALA and SDA decreased with increasing $\delta^{15}N$ values (V, Fig. 6).

Similar to the current study, Koussoroplis et al. (2011) found C_{18} PUFA to decrease with increasing trophic position (depicted also with $\delta^{15}N$ values) in a coastal marine ecosystem.

ARA did not exhibit obvious patterns of trophic enrichment, although high values were detected in fish (4.7 ± 0.9% in vendace, and 6.2 ± 1.8% in smelt), and zooplankton tended to accumulate ARA from spring to fall (II). In contrast, Kainz et al. (2004) found ARA to be highly accumulated with increasing plankton size. However, in their study lakes the pooled macrozooplankton contained mostly large cladocerans, while in the current study the large zooplankton (which also had the highest $\delta^{15}N$ values in plankton) were copepods that generally have lower proportions of ARA than cladocerans. ARA was highly retained in the food web of a coastal Mediterranean lagoon with fish exhibiting ARA levels of >8% in neutral and polar lipids (Koussoroplis et al., 2011). The authors suggested that the very high ARA levels in fish were linked to coping with the stress of the fluctuating temperature, salinity and oxygen in the estuarine environment (Koussoroplis et al., 2011).

The availability of PUFAs for pelagic consumers is ultimately determined by phytoplankton community structure, and the abundant ω3 PUFAs in the studied large lakes were supplied by the cryptophytes and diatoms that dominated the phytoplankton community (I). The mechanism of the trophic enrichment of the $C_{20\text{-}22}$ PUFAs in large lake food webs could include consumers feeding selectively on PUFA-rich food, preferential assimilation and retention of the long-chain PUFAs, and conversely, catabolism or modification of the C_{18} PUFAs by the consumers. Modification of shorter chain PUFA is possible, but the rates are generally negligible (Bell et al., 2007), and thus it is more likely that the 8-fold increase in DHA from seston to fish was the result of selective feeding and other metabolic processes than modification. Copepods feed selectively based on taste or chemical cues, and may avoid detrital particles (Demott, 1986; Butler et al., 1989) that are also low in PUFA. While cladocerans generally are non-selective filter-feeders (excluding the predatory taxa), they efficiently retain PUFA, and especially EPA

and ARA from their diet (Schlechtriem et al., 2006; Taipale et al., 2011). Based on their $\delta^{15}N$ values vendace were presumably feeding on a mixture of copepods and cladocerans, and thus receiving varied proportions of DHA, EPA and ARA from their diet. Dalsgaard & St. John (2004) found PUFA (ALA) to be incorporated into fish tissues faster than SAFA (16:0), and found no evidence of modification of either fatty acid in a tracer study. Furthermore, previous studies indicate that the proportion of DHA in vendace varies among lakes from 13 to 29% (Ågren et al., 1987; Muje et al., 1989; Linko et al., 1992), which suggests that DHA levels are rather influenced by the dietary supply in different lakes than regulated by internal modifications, which should result in similar levels across lakes.

Vendace in the present study had higher ω3:ω6 ratios (5.9 ± 1.2) than previously found for freshwater fish in small lakes (typically < 4) (Ahlgren et al., 1994, 1996; Lau et al., 2011). Littoral and benthic food webs are generally rich in ω6 PUFA with lower ω3:ω6 ratios than pelagic foodwebs, and the high ω3:ω6 ratio and high levels of DHA in vendace may reflect their dependence on pelagic food sources, and especially on DHA-rich copepods. The ω3:ω6 ratios in copepods were consistently higher than in previously studied smaller lakes (Persson & Vrede, 2006; Lau et al. 2011). The glacial relict zooplankton taxa (*Limnocalanus*, *Heterocope*, *Mysis*) could be a very important trophic link in these lakes with high abundance of DHA and EPA (II, V). In addition to providing fish with essential fatty acids, copepods are a rich source of cholesterol, amino acids, vitamins and pigments (van der Meeren et al., 2008).

4 Concluding remarks

The phytoplankton community in the studied lakes was dominated by high quality taxa, cryptophytes and diatoms, and as a result the seston fatty acids were rich in essential PUFAs. Transfer of fatty acids through the pelagic food web was highly selective, and the essential ω3 PUFAs EPA, and particularly DHA, were highly enriched in the upper trophic levels. The mechanisms of PUFA accumulation in zooplankton likely included selective feeding on PUFA-rich resources and the efficient retention of PUFAs from the diet. The abundant copepods together with *Mysis* were responsible for providing the planktivorous fish with high levels of DHA.

As most of the variation in zooplankton fatty acid composition was related to taxonomic identity, the community structure of zooplankton is an important factor determining the PUFAs available for fish. Which taxa are present in lakes is highly influenced by the physical and chemical properties of lakes as well as biological interactions and the biogeographical history (Keller & Conlon, 1994; O´Brien et al., 2004; Leibold et al., 2010). The present data on the copepod *Eudiaptomus* in the lake survey demonstrated that the proportion of DHA can vary 3-fold within this taxa. Furthermore, the parallel responses to water chemistry of the ω3:ω6 ratios among the five zooplankton taxa studied in the large boreal lakes suggest that these differences among lakes may be consistent across the zooplankton community. Most likely water chemistry influenced the fatty acid composition of the zooplankton indirectly by modifying the phytoplankton community structure. Although selective retention of PUFAs by zooplankton may dampen fluctuations in the quality of the basal resources, this study confirms that the resources fish experience likely vary substantially among lakes and seasons.

The selective transfer of PUFAs in the studied pelagic food webs of large boreal lakes produced planktivorous fish highly

enriched in DHA. The efficient accumulation of essential PUFAs in these fish that are a commercially important resource in the region may be disrupted by anthropogenic impacts, such as eutrophication and climate change. Increasing temperatures can reshape phytoplankton community structure by influencing stratification patterns (Winder & Sommer, 2012), and temperature directly affects the metabolic rates and also the fatty acid composition of poikilothermic organisms (Farkas & Herodek 1964; Pruitt, 1990; Gillooly et al., 2001). The cold-adapted, glacial relict zooplankton taxa that were rich sources of EPA and DHA may be particularly vulnerable to changes in ambient temperature.

The changes in precipitation patterns linked to global change may lead to increased nutrient and tOM loading to lake ecosystems (Lepistö et al., 2008; Larsen et al., 2011). Nutrients stimulate primary production and together with high temperatures possibly increase cyanobacteria blooms (Elliot, 2012; Anneville et al., 2015), while tOM can counteract these effects by suppressing primary production by reducing light penetration and favoring flagellate algae of high nutritional quality (Gutseit et al. 2007, Thrane et al., 2014).

As described above the potential impacts of climate change on fatty acid dynamics of lake food webs are not easy to predict. Furthermore, the current study indicates that the impacts may be highly dependent on local characteristics, such as lake morphometry and current water chemistry. Zooplankton fatty acid composition did not change along the nutrient gradient in small and humic lakes, while large clearwater lakes seem to be more vulnerable to changes in water chemistry. The survival and growth of larval fish is very essential fatty acid intensive, and the recruitment success of many fish may be largely determined by survival of these early life-stages (Legget & Deblois, 1994; Tocher, 2010). Thus, changes in the PUFA dynamics of food webs may have comprehensive effects on fish production, and may negatively impact commercial inland fisheries that are usually most concentrated in large lakes.

The importance of fatty acids in trophic dynamics of aquatic ecosystems is becoming increasingly recognized, and the research in the field has progressed tremendously in the last 15-20 years. A combination of field and laboratory studies are still needed to elucidate the relative importance of several potentially limiting and interacting factors, such as temperature, food quantity, and a multitude of food quality parameters (e.g. fatty acids, sterols, amino acids, phosphorus, nitrogen) on the growth of consumers. These studies would provide useful information on how the quality of basal resources can affect the secondary production in lakes, and in turn influence the quantity and quality of resources available for fish. Ultimately, we may be able to explain differences in fisheries production in lakes with varying basal resources, and then build better tools to predict how changes in the system would affect the production and dynamics of fatty acids in the food web.

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Minna Hiltunen

The role of zooplankton in the trophic transfer of fatty acids in boreal lake food webs

The food webs of lakes produce fish that are valuable resources for humans because they are rich in essential polyunsaturated fatty acids (PUFAs). This thesis provides new information on how these PUFAs are transferred through the aquatic food webs, from phytoplankton to fish, and ultimately become available for human consumption. The results indicate that climate change may modify the dynamics of essential PUFAs in lake food webs.

UNIVERSITY OF
EASTERN FINLAND

Publications of the University of Eastern Finland
Dissertations in Forestry and Natural Sciences

ISBN: 978-952-61-1632-7 (printed)
ISSN: 1798-5668
ISBN: 978-952-61-1738-6 (pdf)
ISSN: 1798-5676